NASA TECHNICAL MEMORANDUM NASA TM-75938

EXPERIMENTAL INVESTIGATION OF THE ROLE OF THYROCALCITONIN IN THE PROPHYLAXIS OF DISTURBANCES IN THE WATER-SALT AND MINERAL METABOLISM DURING A 30-DAY HYPOKINESIA

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Translation of "Eksperimental'noye issledovaniye roli tirokal'tsitonina v profilaktike narusheny vodno-solevogo i mineral'nogo obmena pri 30-sutochnoy gipokinesii," Fiziologichesky Zhurnal SSSR im. I.M. Sechenova, Vol. 60, No. 2, 1974, pp. 290-294

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. 20546 NOVEMBER 1979

STANDARD TITLE PAGE

1. Report No.
NASA TM-75938

2. Government Accession No.

3. Recipient's Catalog No.

4. Title and Subtitle
EXPERIMENTAL INVESTIGATION OF THE ROLE OF THYROCALCITONIN IN THE PROPHYLAXIS OF DISTURBANCES OF THE WATER-SALT.....

5. Report Date
November 1979

6. Performing Organization Code

7. Author(s)
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8. Performing Organization Report No.

10. Work Unit No.

11. Contract or Grant No.
NASW-3199

9. Performing Organization Name and Address
Leo Kanner Associates
Redwood City, California 94063

13. Type of Report and Period Covered
Translation

12. Sponsoring Agency Name and Address
National Aeronautics and Space Administration, Washington, D.C. 20546

14. Sponsoring Agency Code

15. Supplementary Notes
Translation of "Eksperimental'noye issledovaniye roli tirokal'tsitonina v profilaktike narusheny vodno-solevogo i mineral'nogo obmena pri 30-sutochnoy gipokinesii," Fiziologichesky Zhurnal SSSR im. P.M. Sechenova, Vol. 60, No. 2, 1974, pp. 290-294 (A74-26557)

16. Abstract

The effect of thyrocalcitonin (TCT) injections on the metabolism of water and electrolytes in free-moving and immobilized chinchilla hares is described. Calcium excretion from immobilized animals was elevated, but normalized in those also receiving TCT injections. TCT also normalized water content and excretion rates.

17. Key Words (Selected by Author(s))

18. Distribution Statement
UNCLASSIFIED Unlimited

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	10	

EXPERIMENTAL INVESTIGATION OF THE ROLE OF THYROCALCITONIN IN THE PROPHYLAXIS OF DISTURBANCES IN THE WATER-SALT AND MINERAL METABOLISM DURING A 30-DAY HYPOKINESIA

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Studies were conducted on the influence of thyrocalcitonin (TCT) /290*
on the metabolism of water, calcium, magnesium, potassium, and sodium, as well as its influence on the condition of skeletal bones of hares under conditions of their free-ranging captivity and during a 30-day restriction of movement. The research was carried out on 98 male chinchilla hares. TCT (bovine, 300 units MRC per mg) was injected subcutaneously two times a day, 50 units per injection, over the period of the experiment.

During hypokinesia there was no change in body excretion of magnesium, potassium, and sodium, but that of calcium progressively increased. Injection of TCT into the motion-restricted animals led to a reduction in calcium excretion, which, however, remained at a higher level than that of the control hares. In addition, TCT had a prophylactic effect on the status of calcium metabolism in the mineralized tissues.

Thyrocalcitonin (TCT), a thyroid iron hormone, is used for prevention of osteoporosis in Itsenko-Cushing's disease, hyperparathyroidism, Paget's disease, etc. (4,5,7). Data are also available on the normalization of calcium metabolism in the bone tissues of rats during prolonged hypokinesia (1). How TCT affects various aspects of water-salt and mineral metabolism during movement-restriction of other types of animals is unknown. The need for carrying out such research is substantiated by data in the literature dealing with the versatility of TCT effects on water and electrolyte metabolism.

The aim of our work was the study of the influence of TCT on water, calcium, magnesium, potassium, and sodium metabolism, as well as

*Numbers in the margin indicate pagination in the foreign text.

its effects on the condition of skeletal bones of hares in free-ranging captivity and during a 30-day restriction of movement (hypokinesia).

Methodology

Four analogous series of experiments were carried out on 98 male chinchilla hares with initial weights of 2500 $\pm$ 200 g. The animals were divided into 4 equal groups: the first was the control group, the second was hypokinetic, the third was the TCT group, and the fourth was hypokinetic + TCT. The 30-day hypokinesia was created by using cramped cages having walls variable in three dimensions (2). TCT (bovine, 300 units MRC/mg (3)) was injected subcutaneously, twice a day at 50 units per injection, into the hares of the third and fourth groups over the entire period of the experiment. The hares were maintained on an adequate standard pelleted ration with the addition of hay and vegetables. Water intake was not limited. On the 2nd, 8th, 15th, 22nd, and 30th days during the experiment, concentrations of sodium and potassium were measured using flame photometry; the concentration of magnesium was measured by atomic absorptiometry, and that of calcium by photoelectrocolorimetric titration; the hares had empty stomachs when tested. Daily measurements of the same electrolytes in the urine and feces were made using atomic absorptiometry. Data on excretion were expressed in milliequivalents per kg weight of the hares. Overall body water was determined by using tritium oxides. For the study of calcium metabolism, Ca^{45} was used, being injected 24 hours /291 before sacrifice (series I), as well as 24 hours before the beginning of experiments (series II), 30 days before (series III), and 60 days before (series IV). After sacrifice, the skeletal bones were removed and processed using the generally accepted method. For morphometric study, identical roentgenograms of the tubular bones were obtained; the thickness of the dense layer and width of the medullary canal in the middle of the diaphysis was determined using an MBS-2 microscope.

Results

In the control hares the concentration of plasma electrolytes during the experiment fluctuated within the following limits: for calcium

-- from 6.29 to 8.06 mEq/liter, magnesium-- from 1.13 to 1.78 mEq/liter, potassium-- from 4.13 to 4.85 mEq/liter, and sodium-- from 141 to 150 mEq/liter. For the hares of all the experimental groups (2nd, 3rd, and 4th), the concentration of potassium and sodium did not differ during the course of the experiment from that found in the control group. The concentration of plasma magnesium during hypokinesia (2nd group) did not change, but that of calcium decreased on the 8th and 15 days (by 6 and 14%, respectively, relative to the control group). Injection of TCT into the hares of the 3rd group led to hypocalcemia on the 8th day (84%, relative to controls, $p < 0.001$), but the concentration of magnesium increased on the 8th, 15th, and 22nd days (118--123% relative to controls). In the motion-restricted animals (4th group) TCT lowered the concentration of calcium on the 8th and 15th days by 6 and 8% ($p < 0.05$) and increased the concentration of magnesium by 11 and 34% ($p > 0.05$).

As can be seen from the illustration, the excretion of calcium from the body (total in urine and feces) during hypokinesia progressively increased: after 7 days--by 10%, 14 days--by 32%, 21 days--by 74%, and 28 days--by 111%, relative to the controls (reliably-- on the 21st and 28th days). Excretion of magnesium, potassium, and sodium did not change. TCT caused reduced excretion of calcium and magnesium in the animals of the 3rd group on the 7th day (81 and 75%) and on the 28th day (88 and 71%, relative to controls). Sodium excretion remained unchanged, but that of potassium increased (164%, relative to controls). With injection of TCT into the motion-restricted animals, excretion of calcium from the body increased: after 14 days--by 27%, 21 days--by 47%, and 28 days--by 24% relative to controls.

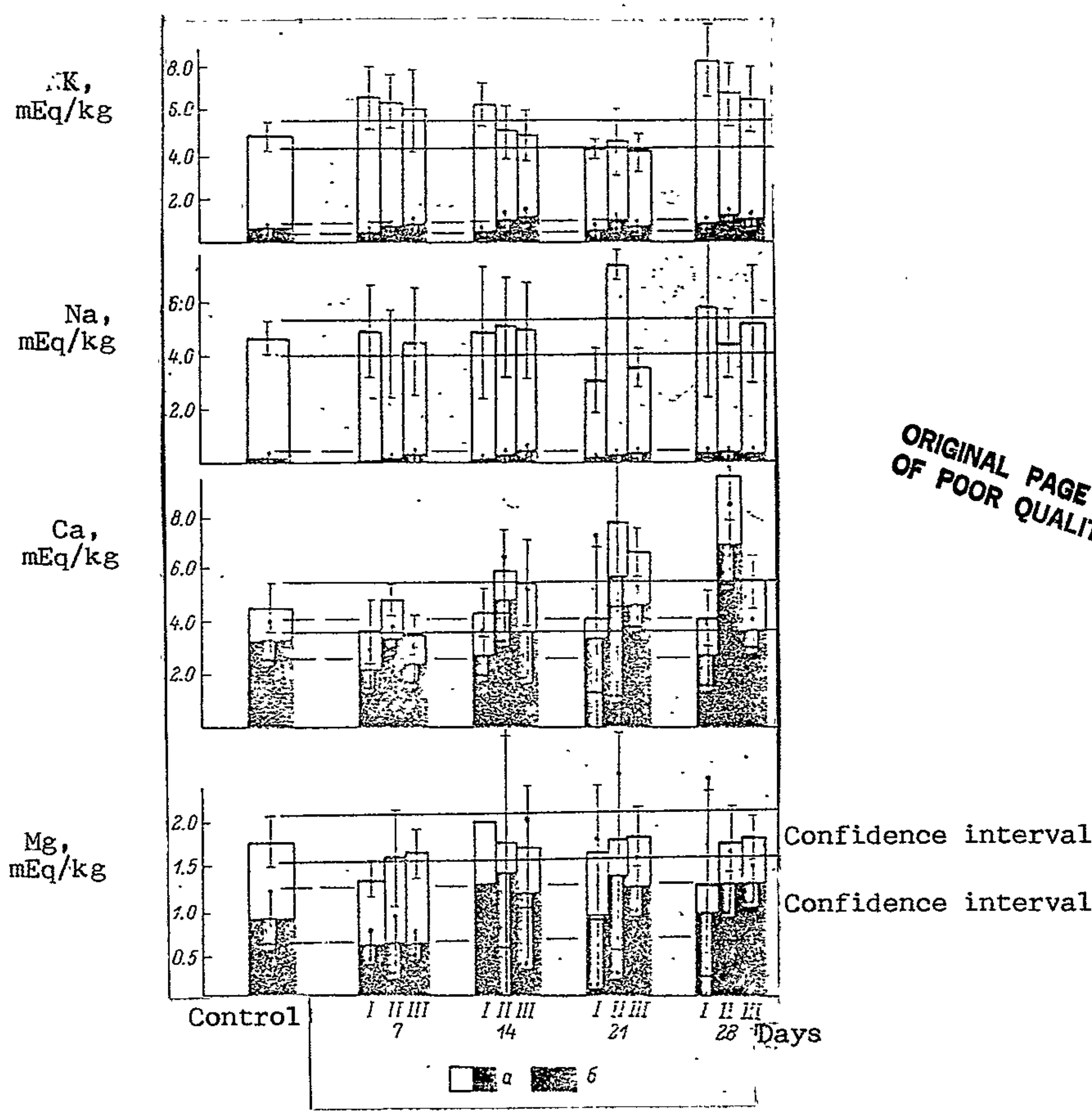
Hence, increased calcium excretion began later and was less pronounced than in the animals with "pure" hypokinesia, particularly on the 28th day, when calcium excretion in the 4th group was 41% lower, relative to data from the 2nd group. Under the influence of hypokinesia and TCT, the total excretion of magnesium did not change, that of sodium decreased on the 21st day (72%), while potassium excretion increased on the 28th day (138% relative to controls), i.e.,

analogously with data from the 3rd group.

The status of water metabolism was judged by urine excretion, overall body water content, and the rate of average daily water loss and replenishment. Urine excretion changed in the same direction for all groups of hares: it decreased on the 2nd day (70--82%) and increased thereafter (119--132% relative to controls). It is possible that the decrease in urine excretion observed at the beginning of the experiment was conditioned by stress and an increased secretion of antidiuretic hormone (6).

Body water content (determined using tritium oxide) in the control animals changed from 634 ± 8.8 ml/kg body weight at the beginning of the experiment to 646 ± 9.9 ml/kg at the termination. With TCT injection, the quantity of water decreased by 3% relative to initial figures ($p < 0.05$). Restriction of movement led to a 7 and 10% increase in water quantity on the 15th and 30th days ($p < 0.001$). With injection of TCT into the motion-restricted animals (4th group), the combined effects of the two influences were observed for 30 days. This amounted to a somewhat smaller increase in water content-- 7% relative to initial. The half-replenishment rate of water in the control animals was 4.6 ± 0.2 days, and increased with injection of TCT to 4.1 ± 0.2 days ($p > 0.05$), and even more as a result of hypokinesia-- to 3.8 ± 0.1 days ($p < 0.01$). With injection of TCT into the motion-restricted animals, the half-excretion period did not differ from that of the controls-- 4.9 ± 0.2 days. Overall water loss in the /292 control hares totaled 100 ± 6.1 ml/kg/day, but during hypokinesia it grew 26% ($p < 0.01$) by day 30. Injection of TCT had no effect on water loss in the free-moving animals (3rd group), but promoted normal water loss in the motion-restricted hares-- 95.5 ± 5.7 ml/kg/day.

Radiometric studies confirmed a sharp decrease in Ca^{45} assimilation (series I) in the calcaneal, femoral, tibial, and parietal bones of the hypokinetic hares (36, 75, 61, and 65%, respectively, relative to controls, $p < 0.05$), which evidenced the general alteration in calcium metabolism. Injection of TCT into the free-moving hares resulted in /293



Excretion of electrolytes in urine and feces
during hypokinesia and injection of thyrocalcitonin
(by body weight in kg)

I--Thyrocalcitonin, II--Hypokinesia, III--hypokinesia+TCT
a--total excretion of electrolytes
b--excretion of electrolytes in feces.

increased inclusion of Ca^{45} into mineralized tissues, particularly the tibiae (155%, $p < 0.05$). During hypokinesia, injection of TCT caused a significant increase in isotope inclusion in the bones: in the calcanei--by 88%, in the femurs--by 39%, in the tibiae--by 222%, and in the parietals--by 78%, compared with data from the 2nd group ($p < 0.05$). Because of the restriction of movement, Ca^{45} resorption in the rapidly-metabolized fractions (series II) of the calcaneal, femoral, and parietal bones was accelerated (isotope content totaled 65, 59, and 75%, respectively, relative to controls, $p < 0.05$). Injection of TCT into the hares of the 3rd group raised radiocalcium resorption in the mineralized tissues an average of 10--30%, while the resorption rate decreased in the hypokinetics (4th group). Thus, there was normalization of Ca^{45} removal from the rapidly-metabolized fraction. As a result of hypokinesia, the resorption rate in the slowly-metabolized fraction of the bones (series III) decreased. Injection of TCT into the animals of the 3rd group led to accelerated Ca^{45} resorption in the mineralized tissues; an analogous effect was observed in the hares of the 4th group. The table shows that resorption of Ca^{45} injected 60 days before the experiment (series IV) increased in the bones of the hypokinetic hares. Resorption of radiocalcium also increased under the influence of TCT (3rd group). Injection of TCT into the motion-restricted animals brought about normalization of Ca^{45} content in the mineralized tissues.

Table 1. Ca^{45} content in the bones of hares during hypokinesia and injection of thyrocalcitonin (data from series IV). Results are given in percents of injected isotope dose.

Groups	Femur			Calcaneus		
	Statistical indices					
	M ± m	p	%vs cntl	M ± m	p	% vs cntrl
Control	0.752±0.057	—	100	0.180±0.013	—	100
Hypokinesia	0.675±0.029	> 0.1	89.8	0.145±0.014	> 0.05	80.5
TCT	0.622±[illegible]	< 0.05	82.9	0.132±0.010	< 0.05	73.2
Hypokinesia+TCT	0.710±0.067	> 0.1	94.5	0.187±0.018	> 0.1	104.3

The thickness of the dense layer of the humeral, femoral, and tibial bones' diaphyses amounted to: 1.94 ± 0.032, 2.31 ± 0.044, 2.82 ± 0.110 mm, while the width of the medullary canals were 4.54 ± 0.086, 6.10 ± 0.097, and 4.74 ± 0.118 mm, respectively. During hypokinesia the dense layer of the diaphyses decreased by 10% in the humeral ($p < 0.02$), by 14.2% in the femoral ($p < 0.01$) and by 18.2% in the tibial bones ($p < 0.02$). Injection of TCT into the free-moving hares resulted in thickening of the diaphyseal walls of the humeral, femoral, and tibial bones (106.3, 109.9, and 111.7% of control values, p 0.05). In the animals kept hypokinetic and also receiving TCT, diaphyseal wall thicknesses were normalized in the humeral and tibial bones (96.7 and 94.3% of control values, $p > 0.1$) and remained almost the same as with "pure" hypokinesia in the femurs (88.7% of control values, $p < 0.02$). Medullary canal widths for animals of all these experimental groups did not actually change.

Thus, we have established that regular changes in water, electrolyte, and mineral metabolism result from 30-day hypokinesia. Injection of TCT has a prophylactic effect primarily on the status of calcium metabolism in mineralized tissues, and also on some water-salt metabolism indices.

REFERENCES

1. Volzhin, A.I., P.V. Vasil'yev, N.N. Uglova, V.E. Potkin, Kosmicheskaya biolog., 3, (1972).
2. Savel'yeva, T.P., V.P. Krotov, Certification No. 278291 (1970).
3. Stekol'nikov, L.I., S.B. Katkovsky, O.M. Tepelina, V.M. Konopatskaya, A. Abdukarimov, T.V. Egorova, Vopr. Med. Khimii, 3, 276, (1969).
4. Band, C.L., et al., Schweiz. med. Wschr., 199, 657, (1969).
5. Bijvoet, O.L.M., Sluys Veer, V. d. S, De Vries, H.R., Kopper, V.A.T., Engl. J. Med., 284, 13, 681, (1971).
6. Fujita, T.H.O., Ohata, M., Endocrinol. Japon, 15, 8, (1968).
7. Gilbert, G.Z., Glaser, G.H., Arch. Neurol., 5, 179, (1961).
8. Fujita, T.H.O., Ohata, M., et al., Endocrinol. Japon., 15, 8, (1968).
9. Keeler, R., Kopp, D.H., McIntoch, H.W., Walker, V., Proc. Can. Fed. Biol. Soc., 13, 78, (1970).
10. Keeler, R., Walker, V., Copp, D.H., Can. J. Physiol. and Pharmacol., 48, 838, (1970).